

Product Data Sheet

Product Name: Ac-Ile-Glu-Pro-Asp-pNA

Cat. No.: GA20580

Chemical Properties

Cas. No. 216757-29-8

Formula $C_{28}H_{38}N_6O_{11}$

M.Wt 634.64

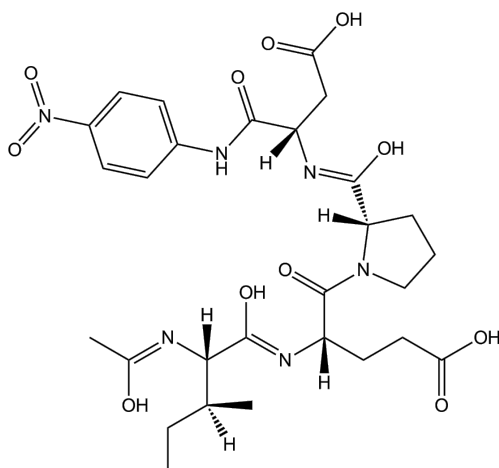
Solubility Soluble in DMSO

Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice. All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

This protocol only provides a guideline, and should be modified according to your specific needs.

1. Solution preparation

(1) Stock solution: Dissolve Ac-Ile-Glu-Pro-Asp-pNA in DMSO to a final concentration of 1 mM.

Note: After unused storage solution is aliquoted, store it at -20°C in the dark to avoid repeated freezing and thawing.

(2) Working solution: Dilute the stock solution with experimental buffer to the required working concentration, for example: 0.2 mM.

Caution: Product has not been fully validated for medical applications. For research use only.

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Note: Please adjust the optimal working concentration according to the actual situation or refer to the literature to set the gradient concentration by yourself. The working solution must be prepared and used immediately.

2. Procedure for Measuring Granzyme B Activity in Cells Using Ac-Ile-Glu-Pro-Asp-pNA [1] (from the literature, for reference only)

(1) Sample aliquoting: Dispense 10µL of cell lysate sample into a 96-well plate.

(2) Add substrate: Add 90µL of Ac-Ile-Glu-Pro-Asp-pNA working solution at a concentration of 0.2 mM.

(3) Optical density measurement: Measure the optical density (OD) at 405 nm at 0 minutes and 90 minutes to determine the change in OD. Use the assay buffer in place of the sample as a blank control.

Note: This protocol provides guidance for measuring granzyme B activity in cells using Ac-Ile-Glu-Pro-Asp-pNA. Adjustments can be made based on other literature and specific experimental requirements.

References:

[1] Chaves-Pozo E, Valero Y, Lozano M T, et al. Fish granzyme A shows a greater role than granzyme B in fish innate cell-mediated cytotoxicity, Front. Immunol. 10 (2019) 2579[J]. 2019.

Background

Ac-Ile-Glu-Pro-Asp-pNA (Ac-IEPD-pNA) is a colorimetric substrate for granzyme B (GzmB) and caspase-8 [1, 2]. Granzyme B and caspase-8 preferentially bind and cleave the Ile-Glu-Pro-Asp (IEPD) peptide sequence, and the catalytic activity of the enzyme is indirectly measured by detecting the release of p-nitroaniline (pNA), which is measured by the optical density (OD) at 405 nm[3]. Granzyme B is a serine protease found in cytotoxic T cells and NK cells, which cleaves and activates several caspases involved in apoptosis[4]. Caspase-8 is a caspase protein that participates in the signal transduction of death receptors of the tumor necrosis factor receptor family and is also essential for the induction of the transcription factor NF-κB[5]. Ac-Ile-Glu-Pro-Asp-pNA is recommended as a potential granzyme B target and the optimal P4-P1 substrate sequence for granzyme B.

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The structural and group characteristics of Ac-Ile-Glu-Pro-Asp-pNA are as follows:

- (1) N-acetyl group (Ac): protects the N-terminus of the amino acid to prevent nonspecific reactions.
- (2) Amino acid sequence (Ile-Glu-Pro-Asp): This specific sequence mimics certain natural substrates and helps to study the specificity of proteases for different substrates.
- (3) p-Nitroaniline (pNA): It is a common chromogenic group that releases a yellow product with light-absorbing properties after hydrolysis, which is convenient for monitoring enzyme reactions through spectral measurement.

References:

- [1]Ewen C, Kane K P, Shostak I, et al. A novel cytotoxicity assay to evaluate antigen-specific CTL responses using a colorimetric substrate for Granzyme B[J]. Journal of immunological methods, 2003, 276(1-2): 89-101.
- [2]Almeida S, Domingues A, Rodrigues L, et al. FK506 prevents mitochondrial-dependent apoptotic cell death induced by 3-nitropropionic acid in rat primary cortical cultures[J]. Neurobiology of disease, 2004, 17(3): 435-444.
- [3]Chaves-Pozo E, Valero Y, Lozano M T, et al. Fish granzyme A shows a greater role than granzyme B in fish innate cell-mediated cytotoxicity, Front. Immunol. 10 (2019) 2579[J]. 2019.
- [4]Rousalova I, Krepele E. Granzyme B-induced apoptosis in cancer cells and its regulation[J]. International journal of oncology, 2010, 37(6): 1361-1378.
- [5]Chun H J, Zheng L, Ahmad M, et al. Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency[J]. Nature, 2002, 419(6905): 395-399.

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